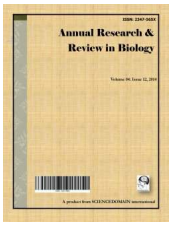

Annual Research & Review in Biology

13(6): 1-8, 2017; Article no.ARRB.34349
ISSN: 2347-565X, NLM ID: 101632869

Evaluation of Antioxidant and Free Radical Scavenging Activity of *Tagetes patula*

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Authors' contributions

This work was carried out in collaboration between both authors. Author DK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YV managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/34349
Editor(s):
(1) Bechan Sharma, Department of Biochemistry, University of Allahabad, Allahabad, India.
(2) George Perry, University of Texas at San Antonio, USA.
Reviewers:
(1) Vanessa de Andrade Royo, Universidade Estadual de Montes Claros, Brazil.
(2) Eliton da Silva Vasconcelos, Federal University of São Carlos, UFSCar, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/19782

Original Research Article

Received 24th May 2017
Accepted 11th June 2017
Published 30th June 2017

ABSTRACT

In present study, the preliminary screening of *Tagetes patula* contains many phytochemicals. These phytochemicals are able to reduce the oxidative stress in living organisms under adverse conditions. *T. patula* contains high ratio category of polyphenolic compounds such as phenol and flavonoids. Antioxidant activity of extracts was expressed as percentage of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals inhibition and IC_{50} values (µg/mL) ranged from 11.86 to 16.06%. The total phenolic content ranged from 30.26 to 80.08 mg/g of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentration varied from 30.00 to 65.00 mg/g, expressed as quercetin equivalents. In this study, phenolic content was quite higher in leaves as compared to flavonoids in flowers. The objective of this study to determined the antioxidant activity of *Tagetes patula*.

Keywords: Antioxidants; DPPH; flavonoids; phenol; Tagetes patula.

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ABBREVIATIONS

(TPC) : Total phenolic content
(TFC) : Total flavonoid content
(DPPH) : 1,1-diphenyl-2-picryl-hydrazyl
(BHT) : Butylatedhydroxytoluene
(mg) : Milli gram
(µg) : Micro gram
(g) : Gram
(mL) : Milli litter
(IC) : Inhibition concentration
(rpm) : Revolutions per minute
(mM) : Milli molar
(nm) :Nanometer
(GAE) :Gallic acid
(QE) :Quercetin
(%) :Percentage

1. INTRODUCTION

India is known as the garden of medicinal plants, which are the rich source of pharmaceutical agent for the prevention of the diseases. *Tagetes patula* is an aromatic annual herb belongs to the family *Asteraceae* [1]. North and South America is the origin of the *Tagetes* genus especially in Mexico. During the 16 century, marigold plants spread all over world. Now days, it is cultivated in approximately all the Asian countries. In India mainly yellow or orange flowers plant of marigold are traditionally used as spices, tea and medicine. The fresh and dried plant can be made into tea, tincture, ointments and cream. The active constituents of the plant are carotenoids (hence the orange colour), essential oils, flavonoids, sterols, tannins, saponins, triterpene alcohols, polysaccharides [2]. Presence of the secondary metabolites is responsible for the specific fragrance in *Tagetes* plant. Due to the presence of secondary metabolites plants have played important role in biological and pharmacological activities such as anti-oxidative, anti-allergic, anti-carcinogenic, antimicrobial and hypoglycemic etc. [3]. Antioxidants are free radical capturing substance, which is already present in plants. It may protect cells from the damage of unstable molecules *i.e* free radicals [4,5,3]. Antioxidant may be either the natural ones or synthetic ones [6,7]. Natural antioxidants, which are obtained from plant having greater benefit in comparison to the synthetic antioxidant: butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate and tertiary butyl hydroquinone [8,6,7]. The main aromatic hydrocarbon component present in it is terpenes. *T. patula* exhibits anti-inflammatory, antibacterial, antifungal insecticidal, astringent, diuretic, skin disorders, and hepatic disorder activity, in addition to antioxidant activities associated with free radical scavenging. The aim of this study is estimation of total phenol, flavonoid content and antioxidant activity of *Tagetes patula*.

2. MATERIALS AND METHODS

2.1 Plant Material

The freshly leaves and flowers of *Tagetes patula* L., were collected during the month of November –February 2015 from the Allahabad.

2.2 Chemical

Butylated hydroxytoluene (BHT), Folin–Ciocalteu' phenol reagent, the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH), gallic acid, and sodium carbonate (Na_2CO_3) were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). Acetone and methanol were purchased from Merck. All other chemicals used were of analytical grade.

2.3 Extraction of the Plant Material

Fresh plant sample (10 g) was collected and washed properly under running water to remove the dust. Samples were ground with 80% alcohol for 20 minute.

2.4 Phytochemical Screening

The aliquots of crude extract of *Tagetes patula* were used for the preliminary analysis [9].

2.5 Determination of Total Phenolic Content

The total phenol content of marigold samples (flowers and leaves) were determined by the method of [10,11] with slight modification. Each extracted sample was transferred and made up the volume with 3 ml distilled water, followed by Folin ciocalteau reagent 0.5 ml (1:1 with water). It was mixed for 3 min. then 2 mL of Na_2CO_3 (20%) was added and incubated for a further 30 min. at room temperature in dark. A dark blue colour was developed and read at 650 nm. Gallic acid was used as the standard for the formation of calibration curve to the calculation of total phenol content in mg/g.

2.6 Determination of Total Flavonoid

The total flavonoid content of methanol extracted sample was determined by the aluminium chloride ($AlCl_3$) method of [12]. Quercetin was used as the standard for the calculation of the calibrated curve. The different aliquots of sample and standard were taken, and made up the volume to 1 ml with methanol followed by $AlCl_3$ (0.1 mL) and sodium acetate (0.1 mL). The reaction mixture was diluted by 1 mL distilled water and the absorbance was measured at 510 nm.

2.7 Evaluation of DPPH Radical Scavenging Assay

Free radical scavenging activity of marigold plant was carried out by the method [13,14]. Prepared the 0.1 mM of DPPH solution and plant extract with different concentrations (50 µg and 100 µg). The reaction mixture contained equal amount of DPPH solution and plant samples, incubated for 30 min. at room temperature. The absorbance was recorded at 517 nm against the BHT. Free radical scavenging was calculated by using following equation:

$$\text{Percentage inhibition} = (A_{DPPH} - A\ \text{sample})/A_{DPPH \times 100}$$

2.8 Statistical Analysis

Statistical analysis was done in a triplicate manner to analyze the significance variances at 95% confidence limit by using statpac-16.0.15 version.

3. RESULTS

3.1 Phytochemical Screening

Preliminary analysis of the *Tagetes* extract showed the major classes of phytochemical. Major classes of phytochemical present in *Tagetes* extract were phenols, flavonoids, saponin, alkaloids, terpenoids, and steroids [9].

3.2. Total Phenolic Content

The present study revealed the total phenol content of the leaves and flowers of *T. patula* expressed in term of gallic acid. The yield of gallic acid was found 30 GAE/g for flowers and for leaves it was 80 GAE/g respectively as shown in Fig. 1. The total phenolic contents were calculated by using the following linear equation based on calibration curve of gallic acid:

$y = 1.378x + 0.207$ $R^2 = 0.994$ for flowers and $y = 1.378x + 0.207$ $R^2 = 0.994$ for leaves. The total phenolic content test was found to be significant at the 5% level.

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action [15].

3.3 Total Flavonoid Content

The flavonoid content in the flowers and leaves extract was expressed in terms of quercetin equivalent (the standard curve equation $y = 0.915x + 0.591$ $R^2 = 0.991$) for flowers and standard curve equation $y = 2.401x + 0.495$ $R^2 = 0.997$ for leaves. The total phenolic content test was found to be significant at the 5% level. The flavonoid content was found in range30-65quercetin equivalents per gram of extract respectively in leaves and flowers as shown in Fig. 2.

3.4 DPPH Radical Scavenging Activity

The free radical scavenging activity of *T. patula* of flower and leaf extracts were compared with standard antioxidant BHT in this study. The results were expressed as inhibition (%) as shown in Table 2. The strong DPPH free-radical scavenging activity present in leaves were high

Table 1. Preliminary phytochemicals analysis of *T. patula* extract

Phytochemical test	Name of the test	Flower extract	Leave extract
Saponin	Froth test	Absent (--)	Absent (--)
Alkoloids	Meyer's & Wagner's	Present (++)	Present (++)
Flavonoids	Shinoda test	Present (++)	Present (++)
Terpenes	Salkowski test (modified)	Present (++)	Present (++)
Steroids	Salkowski	Present (++)	Present (++)
Proline	Ninhydrin	Present (++)	Present (++)

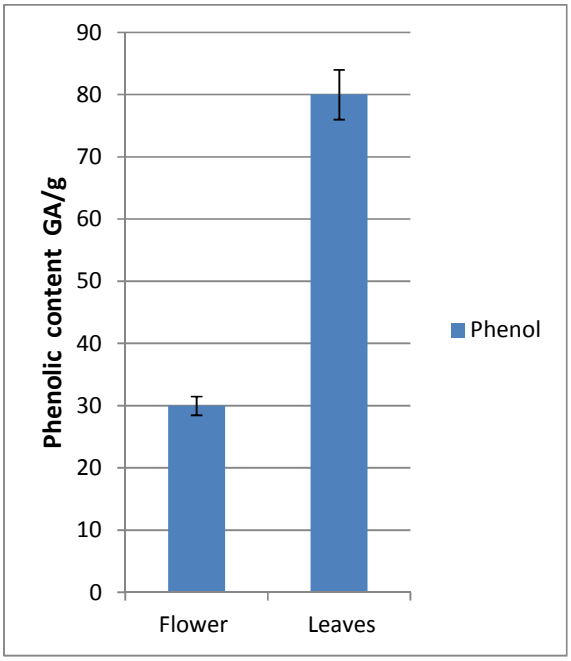

Fig. 1. Total phenol content of different extracts of *T. patula*

This Fig. 1 depicted that the leaf has higher phenolic content as compared to the flower. The value of phenolic content present in leaves and flower is 80 GA/g and 30 GA/g

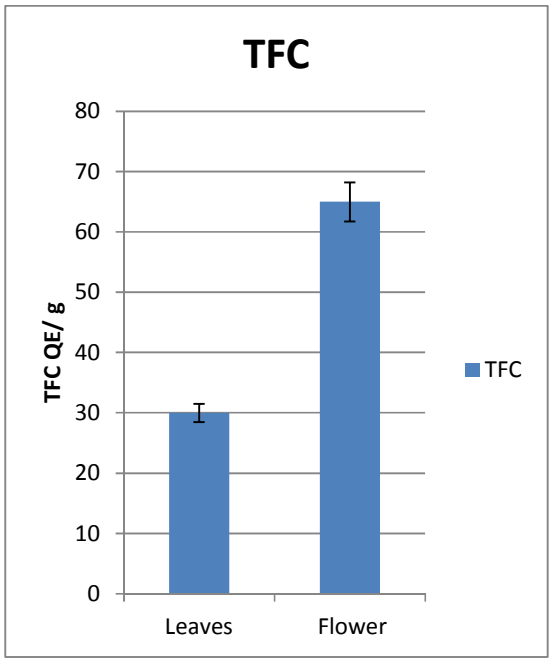

Fig. 2. Total flavonoid content of different extracts of *T. patula*

This Fig. 2 depicted that the flower has higher flavonoids content as compared to the leaf. The value of flavonoids content present in leaves and flower is 30 QE/g and 65 QE/g

as compared to the flower extracts. The extracts showed good antioxidant activity, but all tested concentrations of standard had higher scavenging activities as compared to the samples. It is usually expressed as IC_{50} value, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. Lower IC_{50} value indicates high antioxidant activity [16].

Table 2. Evaluation of DPPH free-radical scavenging activity of methanolic plant extracts of *T. patula* with respect of standard BHT

Concentration(µg/ml)	% of inhibition		
	Flowers	Leaves	BHT
50µg/ml	12.39629	11.85476	3.27007
100µg/ml	16.0588	15.66478	8.966667

DPPH % inhibition test was found to be significant at the 5% level.

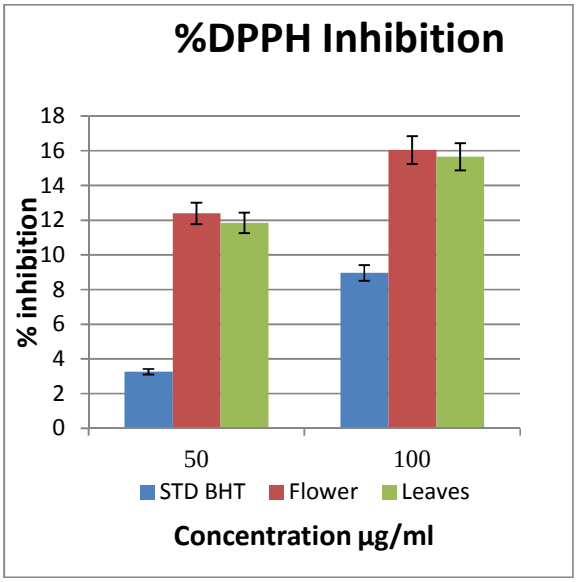

Fig. 3. Evaluation of DPPH free-radical scavenging activity of methanolic plant extracts of *T. patula* with respect to standard BHT. Leaves show higher antioxidant activity as compare to flowers

4. DISCUSSION

In preliminary screening, result of *T. patula* had revealed the presence of polyphenolic compounds in the samples. The classes of secondary metabolites present in *T. patula* were: phenol, flavonoids, alkaloids, terpenoids and steroids. One of the categories of secondary metabolite saponin was not present in the plants as shown in Table 1.Secondary metabolites are important sources of natural antioxidants [17]. The synthesis of secondary metabolites in different plant species is mainly controlled by genetics and could be stimulated by geographical and climatic factors such as temperature, precipitation, UV lights, light intensity, altitude, latitude and longitude, etc [18]. Natural antioxidant reduces the risk of disorders like neurodegenerative diseases, ulcer, cancer, hepatotoxic diseases and AIDS (HIV) infection [14,19,20]. Antioxidants through their scavenging power are useful for the management of those diseases [17]. The present study mainly focuses on antioxidant activity determination of Phenol, Flavoniods and total activity of DPPH. The study shows that 30-80 GAE/g of Total phenolic content (TPC) and Total flavonoid content (TFC) of 30-65 QE/g was found in *T. patula.* TPC was higher in the leaves and Total flavonoid content (TFC) was higher in flowers.

TFC in *T. patula* ranged 4.63 to 25.13% (w/w) [21]. The highest TPC and TFC in *T. erecta* flower were reported 62.33 mg GAE/ g and 97.00 mg QE/ g respectively [22].

The phenolic compounds present in plants are known for their antioxidant activity. Many studies have shown that polyphenols and their bioactivities contribute significantly to the antioxidant activity and act as highly effective metal chelating, lipoxygenase inhibitors and free radical scavengers which are mainly due to their redox properties. It can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or

decomposing peroxides [23-27,14,21]. Flavonoids, inhibit hydrolytic and oxidative enzymes, anti-inflammatory action, antiviral, reduce blood-lipid and glucose, chelation of metal ions and to enhance human immunity [28-30]. Antioxidant properties, especially radical scavenging system activity, are very important due to the deleterious role of food and biological systems [31,32].

The antioxidant activity of extracts was based on their ability to trap DPPH radical. DPPH method is sensitivity way to determine the antioxidant activity of plant extracts [23]. DPPH free radical scavenging activity of *T. patula* extracts were compared with standard BHT in this study. The extracts showed the good antioxidant activities in different manner, but all tested concentrations of standard had higher scavenging activities as compared to the samples. This variation observed between scavenging activities depends on the leaves and flowers of plant used for the study. This difference could be attributed to unequal distribution of antioxidant molecules such as polyphenol, flavonoids in different parts of the plant [23]. DPPH is fast, easy and reliable method. DPPH is a stable, synthetic radical that does not disintegrate in water, methanol, or ethanol. The free radical scavenging activities of extracts depend on the presence of antioxidant compounds because it has a property to donate hydrogen atom and form structural conformation of DPPH components [33]. Phenolic hydroxyls and carboxylic acid moieties have been recognized to function as electron or hydrogen donor. Thus, the DPPH radicals scavenging activity of the extracts may be mostly related to the phenolic hydroxyls and carboxylic acid groups [17,34,35]. The present study revealed that the leaves and flower of *T. patula* contain significant amounts of phenol and flavonoid. The objective of this study was to get information of the amount of phenolics and flavonoids in different parts of *T. patula.* The flowers and leaves extract of *T. patula* exhibited the good antioxidant activity. Flowers and leaves of *T. patula* can be used as a natural source of antioxidants for medicinal use.

5. CONCLUSION

The present study indicates that the *Tagetes sp.* are potential source of antioxidant activity leads by secondary metabolites. This study suggests that *Tagetes patula* leaves are rich in phenolic compounds and flowers have higher content of flavonoids, vice versa. Due to the good source of antioxidant activity the different parts of the *Tagetes* plants can be used as prevent progression of many diseases. However, further studies are needed to evaluate *in-vivo* antioxidant activity and isolation and characterization of an individual active compound from plant extracts which justify the use of the traditional medicine. Determination of antioxidant compound in plant extracts will use to developed new drugs in pharmaceutical fields and in cosmetic world.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI: 10.1007/s13197-011-0389

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19782